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Two new *Botryosphaeria* (Botryosphaeriales, Botryosphaeriaceae) species in China

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Abstract

Five ascomycetous strains were isolated from dead branches and leaves of *Salix* (Salicaceae) and *Osmanthus fragrans* (Oleaceae), respectively. BLAST searches with ITS sequences in GenBank suggested a high degree of similarity to *Botryosphaeria dothidea*. To accurately identify these strains, we further analysed their morphological characteristics of asci, ascospores, all conidiophore cells and conidia. Phylogenetic relationships, based on ITS, *rpb2*, *tef1* and *tub2* gene sequences, confirmed our strains represented two novel species, which are introduced here as *B. salicicola* and *B. osmanthuse* **spp. nov.**

Keywords

Ascomycetes, molecular analyses, morphology, new species, new woody host

Introduction

The genus *Botryosphaeria* (Botryosphaeriales, Botryosphaeriaceae) was established by Cesati and Notaris (1863) and is widely distributed throughout many geographical and climatic regions of the world, with the exception of polar regions (Phillips et al. 2013). Species of *Botryosphaeria* are reported in many woody plants as endophytes, saprobes and pathogens (Crous et al. 2006; Liu et al. 2012; Phillips et al. 2013; Ariyawansa et al. 2016; Dissanayake et al. 2016; Slippers et al. 2017). Some species of *Botryosphaeria* are aggressive pathogens that pose a significant threat to agricultural and forest ecosystems (Slippers and Wingfield 2007). *Botryosphaeria dothidea* is known to cause serious diseases,

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such as Apple ring rot (Slippers and Wingfield 2007; Marsberg et al. 2017). Moreover, according to the database of the common names of plant diseases in Japan, 14 species of the genus *Botryosphaeria* cause diseases on 30 plant species (Yukako et al. 2021).

Botryosphaeria has been considered as one of the hot topics in fungal taxonomy for a long time, based on its universality, including areas and hosts (from 1863 to 2022) (Cesati and Notaris 1863; Shoemaker 1964; Pennycook and Samuels 1985; Slippers et al. 2004; Slippers and Wingfield 2007; Liu et al. 2012; Phillips et al. 2008, 2019; Xu et al. 2015; Ariyawansa et al. 2016; Zhou et al. 2016, 2017; Li et al. 2018, 2020; Vu et al. 2019; Chen et al. 2020; Chu et al. 2021; Yukako et al. 2021). More than 300 species epithets are listed in MycoBank (https://www.mycobank.org, 17 October 2022), but only about 7% of *Botryosphaeria* species currently have associated DNA sequences data. In the past, species in *Botryosphaeria* were defined, based on morphological characters alone or on host association, but studies have shown these are inadequate characters to identify species (Shoemaker 1964; Pennycook and Samuels 1985; Slippers et al. 2004). With the advent of DNA sequencing methods, the nomenclature and identification of *Botryosphaeria* species have significantly improved (Phillips et al. 2013).

Some species of *Botryosphaeria* are aggressive pathogens in China, mainly distributed in the southwest, such as *B. fabicerciana*, *B. fujianensis*, *B. fusispora*, *B. kuwatsukai*, *B. dolichospermatii*, *B. pseudoramosa* and *B. wangensis* as shown in Table 4. In this study, five strains were isolated during surveys of fungi on new woody hosts (Salicaceae and Oleaceae) in Guizhou and Guangxi Provinces, China. Combining morphology and phylogenetic analyses, these isolates represent two novel *Botryosphaeria* species, which are described and illustrated here. The discovery of new species within this genus is important to help researchers better understand the diversity and ecology of *Botryosphaeria*.

Materials and methods

Sampling, fungal isolation and morphological observation

Fungi were isolated from dry branches of *Salix* (Salicaceae) and diseased leaf pieces of *Osmanthus fragrans* (Oleaceae) collected in forest parks in Guizhou and Guangxi Provinces, China, respectively. Samples were placed in envelopes and returned to the laboratory as described by Senanayake et al. (2020). Fruiting bodies (including asci, ascospores, conidiophore cells and conidia) on natural substrates were observed using a Zeiss Scope 5 compound microscope Axioscope 5 (Carl Zeiss Microscopy GmbH, Jena, Germany) with the microscope techniques of differential interference contrast light (DIC) and photographed using an AxioCam 208 colour (Carl Zeiss Microscopy GmbH, Jena, Germany) camera and saved as JPG files. Approximately 30 measurements of new species were made of each feature using the ZEN 3.0 (blue edition) (Jena, Germany) software.

Pure cultures were obtained using a single spore isolation method as described in Senanayake et al. (2020). The germinated spores were transferred to fresh potato dextrose agar (PDA) plates and incubated at 25 °C for 14 days. Type specimens were deposited in the Herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University

(**HGUP**). Ex-type cultures were deposited in the Culture Collection at the Department of Plant Pathology, Agriculture College, Guizhou University, P.R. China (**GUCC**). Taxonomic information of the new species was submitted to MycoBank (www.mycobank.org).

DNA extraction, PCR and sequencing

Mycelium growing on PDA for seven days was scraped off with a sterile scalpel. Total DNA was extracted with a (Biomiga#GD2416, San Diego, California, USA) BIOMIGA Fungus Genomic DNA Extraction Kit (GD2416) following the manufacturer's protocol. Four loci (ITS, *rpb2*, *tef1* and *tub2*) were amplified with the respective forward and reverse primers (Table 1). PCR cycling conditions were followed according to Yukako et al. (2021). For ITS: initial denaturation (94 °C, 5 min), 40 cycles of amplification (denaturation 94 °C, 45 s; annealing 48 °C, 30 s; and extension 72 °C, 90 s) and final extension (72 °C, 2 min); for *tef1*: initial denaturation (94 °C, 5 min), 40 cycles of amplification (denaturation 94 °C, 30 s; annealing 52 °C, 30 s; and extension 72 °C, 45 s) and final extension (72 °C, 2 min); for *tub2*: initial denaturation (94 °C, 5 min), 40 cycles of amplification (denaturation 94 °C, 30 s; annealing 52 °C, 30 s; and extension 72 °C, 60 s) and final extension (72 °C, 2 min); and for *rpb2*: initial denaturation (95 °C, 5 min), touch-down amplification (5 cycles of 95 °C for 45 s, 60 °C for 45 s and 72 °C for 120 s; 5 cycles of 95 °C for 45 s, 58 °C for 45 s and 72 °C for 120 s; and 30 cycles of 95 °C for 45 s, 54 °C for 45 s and 72 °C for 120 s) and final elongation at 72 °C for 8 min. PCR products were sequenced by SinoGegoMax (Beijing, China).

Table 1. Primers used in this study.

Used genes	Primer	Direction	Sequence (5'–3')	Reference
tef1	EF1-688	Forward	CGGTCACTTGATCTACAAGTGC	Alves et al. (2008)
	EF1-1251	Reverse	CCTCGAACTCACCAGTACCG	
ITS	ITS1	Forward	TCCGTAGGTGAACCTGCGG	White et al. (1990)
	ITS4	Reverse	TCCTCCGCTTATTGATATGC	
tub2	BT-2a	Forward	GGTAACCAAATCGGTGCTGCTTTC	Glass and Donaldson (1995)
	BT-2b	Reverse	ACCCTCAGTGTAGTGACCCTTGGC	
rpb2	fRPB2-5f2	Forward	GATGATAGAGATCATTTTGG	Liu et al. (1999)
	fRPB2-7cR	Reverse	CCCATAGCTTGTTTACCCAT	

Phylogenetic analyses

Newly-generated sequences were deposited in GenBank. All the taxa used in the phylogenetic analyses are provided in Table 2. These sequences were compared with the GenBank database using the Basic Local Alignment Search Tool (BLAST) and available sequences of species in the genus containing ex-type or representative isolates were downloaded from GenBank and previous publications (Li et al. 2018, 2020; Vu et al. 2019; Chen et al. 2020; Chu et al. 2021; Yukako et al. 2021). Alignments for the individual locus matrices were generated with the online version of MAFFT v. 7.307 (Katoh et al. 2019). Ambiguous sequences at the start and the end were deleted and

the alignments edited with MEGA6 (Tamura et al. 2013) for maximum alignment and minimum gaps. Sequence matrix v. 1.7.8 was used to concatenate the aligned sequences (Vaidya et al. 2011). *Neoscytalidium dimidiatum* (CBS 145.78 and CBS 251.49) and *Cophinforma atrovirens* (MFLUCC 11-0425 and MFLUCC 11-0655) were used as outgroup. Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI) were used to place the newly-discovered strains into a phylogenetic framework and estimate phylogenetic relationships with other *Botryosphaeria* spp.

ML analysis was performed using IQ-TREE (Nguyen et al. 2015; Trifinopoulos et al. 2016) on the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at, 17 October 2022). The MP analysis was implemented to test the discrepancy of the ITS, *rpb2*, *tef1* and *tub2* sequence datasets with PAUP v. 4.0b10 (Swofford 2002). Gaps were treated as missing data, which were interpreted as uncertainty of multistate taxa. Phylogenetic trees were generated using the heuristic search option with tree bisection re-connection (TBR) branch swapping. "Maxtrees" was set to 5000, the tree length (TL), consistency index (CI), homoplasy index (HI), retention index (RI) and rescaled consistency index (RC) were calculated. Bayesian Inference analysis was made with MrBayes 3.2.6 (Ronquist et al. 2012) based on a best substitution model for ITS: GTR+G, *rpb2*: K2P+I, *tef1*: HKY+G and *tub2*: HKY+G. BI was performed using six Markov Chain Monte Carlo runs for 5,000,000 generations, sampling every 1000 generations. The first 25% resulting trees were discarded as burn-in phase of each analysis.

MP, ML bootstrap support values greater than 70% and BI posterior probability values greater than 0.90 were denoted at the nodes and separated by "/". Bootstrap values less than 70% and BI posterior probability values less than 0.90 were labelled with "_".

Results

The MP, ML and Bayesian analyses resulted in trees with similar topologies and the MP tree is shown in Fig. 1. The combined data matrix of ITS–*rpb2*–*tef1*–*tub2* consisted of 1805 characters (ITS: 466, *rpb2*: 716, *tef1*: 286 and *tub2*: 337), of which 1579 characters were constant and 13 variable characters were parsimony uninformative. Maximum Parsimony analysis of the remaining 213 parsimony informative characters produced a tree with the following parameters: TL = 291; CI = 0.862; HI = 0.137; RI = 0.931; and RC = 0.803.

In the phylogenetic tree (Fig. 1), the isolates from this study formed two distinct, well-supported clades and, thus, were considered to represent two previously unknown species. *Botryosphaeria osmanthuse* GUCC 21433, GUCC 21433.1 and GUCC 21433.2 without the DNA base differences in four loci amongst strains (ITS, *rpb2*, *tef1* and *tub2*) form an independent branch with strong support (ML = 85, PP = 0.94) sister to *B. puerensis*. *Botryosphaeria salicicola* (GUCC 21230 and GUCC 21230.1) clustered sister to *B. corticis*, *B. fabicerciana*, *B. fusispora*, *B. fujianensis*, *B. kuwatsukai* and *B. rosaceae*, although with weak-supports (ML = 75). These two novel taxa were also supported by DNA base pair differences (Table 3).

Table 2. Taxa used for molecular phylogenetic analyses and their GenBank accession numbers. (T) = ex-type strains.

Species	Strain	Host	Country	GenBank accession numbers			
				ITS	*tef1*	*tub2*	*rpb2*
Botryosphaeria agaves	CBS 133992[T]	*Agave* sp.	Thailand	JX646791	JX646856	JX646841	N/A
B. agaves	MFLUCC 10-0051	*Agave* sp.	Thailand	JX646790	JX646855	JX646840	N/A
B. auasmontanum	CMW 25413[T]	*Pinus* sp.	Namibia	KF766167	N/A	N/A	N/A
B. corticis	CBS 119047[T]	*Vaccinium corymbosum*	USA	DQ299245	EU017539	EU673107	N/A
B. corticis	ATCC 22927	*Vaccinium* sp.	USA	DQ299247	EU673291	EU673108	N/A
B. dothidea	CBS 115476[T]	*Prunus* sp.	Switzerland	AY236949	AY236898	AY236927	N/A
B. dothidea	CBS 110302	*Vitis vinifera*	Portugal	AY259092	AY573218	EU673106	N/A
B. fabicerciana	CBS 127193[T]	*Eucalyptus* sp.	China	HQ332197	HQ332213	KF779068	N/A
B. fabicerciana	CMW 27121	*Eucalyptus* sp.	China	HQ332198	HQ332214	KF779069	N/A
B. fujianensis	CGMCC 3.19099[T]	*Vaccinium uliginosum*	China	MH491973	MH491977	MH562330	N/A
B. fujianensis	BJFUCC 180226-3	*Vaccinium uliginosum*	China	MW251380	MW251388	MW251379	N/A
B. fusispora	MFLUCC 10-0098[T]	*Entada* sp.	Thailand	JX646789	JX646854	JX646839	N/A
B. fusispora	MFLUCC 11-0507	*Caryota* sp.	Thailand	JX646788	JX646853	JX646838	N/A
B. guttulata	CGMCC3.20094[T]	N/A	China	MT327839	MT331606	N/A	N/A
B. guttulata	GZCC 19-0188	N/A	China	MT327833	MT331601	N/A	N/A
B. kuwatsukai	CBS 135219[T]	*Malus domestica*	China	KJ433388	KJ433410	N/A	N/A
B. kuwatsukai	LSP 5	*Pyrus* sp.	China	KJ433395	KJ433417	N/A	N/A
B. dolichospermatii	CGMCC 3.19096[T]	*Vaccinium uliginosum*	China	MH491970	MH491974	MH562327	N/A
B. dolichospermatii	CGMCC 3.19097	*Vaccinium uliginosum*	China	MH491971	MH491975	MH562328	N/A
B. minutispermatia	GZCC 16-0013[T]	Dead wood	China	KX447675	KX447678	N/A	N/A
B. minutispermatia	GZCC 16-0014	Dead wood	China	KX447676	KX447679	N/A	N/A
B. osmanthuse	**GUCC 21433[T]**	**GUCC 21433**	**China**	**OL854215**	**OP650906**	**OP669376**	**OP650903**
B. osmanthuse	**GUCC 21433.1**	***Osmanthus fragrans***	**China**	**OL854216**	**OP650907**	**OP669377**	**OP650904**
B. osmanthuse	**GUCC 21433.2**	***Osmanthus fragrans***	**China**	**OL854217**	**OP650908**	**OP669378**	**OP650905**
B. pseudoramosa	CERC 2001[T]	*Eucalyptus hybrid*	China	KX277989	KX278094	KX278198	MF410140
B. pseudoramosa	CERC 2983	*Melastoma sanguineum*	China	KX277992	KX278097	KX278201	MF410143
B. puerensis	CSF6052 T	*Eucalyptus urophylla*	China	MT028569	MT028735	MT028901	MT029057
B. qingyuanensis	CERC 2946[T]	*Eucalyptus hybrid*	China	KX278000	KX278105	KX278209	MF410151
B. qingyuanensis	CERC 2947	*Eucalyptus hybrid*	China	KX278001	KX278106	KX278210	MF410152
B. quercus	MFLUCC:14-0459 T	*Quercus* sp.	Italy	KU848199	N/A	N/A	N/A
B. ramosa	CBS 122069[T]	*Eucalyptus camaldulensis*	Bell Australia	EU144055	EU144070	KF766132	N/A
B. ramosa	CGMCC 3.18004	*Acacia* sp.	China	KX197073	KX197093	KX197100	N/A
B. rosaceae	CGMCC 3.18007[T]	*Malus* sp.	China	KX197074	KX197094	KX197101	N/A
B. rosaceae	CGMCC 3.18008	*Amygdalus* sp.	China	KX197075	KX197095	KX197102	N/A
B. salicicola	**GUCC 21230[T]**	***Salix***	**China**	**OL854218**	**OP669379**	**OP750032**	**N/A**
B. salicicola	**GUCC 21230.1**	***Salix***	**China**	**OL854219**	**OP669380**	**OP750033**	**N/A**
B. scharifii	CBS 124703[T]	*Mangifera indica*	Iran	JQ772020	JQ772057	N/A	N/A
B. sinensia	CGMCC 3.17722[T]	*Populus* sp.	China	KT343255	N/A	N/A	N/A
B. tenuispora	MUCC 2900	*Aucuba japonica*	Japan	LC585276	LC585148	LC585172	N/A
B. tenuispora	MUCC 237[T]	*Leucothoe fontanesiana*	Japan	LC585278	LC585150	LC585174	LC585196
B. wangensis	CERC 2298[T]	*Cunninghamina deodara*	China	KX278002	KX278107	KX278211	MF410153
B. wangensis	CERC 2299	*Cunninghamina deodara*	China	KX278003	KX278108	KX278212	MF410154
Cophinforma atrovirens	MFLUCC 11-0425 T	*Eucalyptus* sp.	Thailand	JX646800	JX646865	JX646848	N/A
C. atrovirens	MFLUCC 11-0655	*Eucalyptus* sp.	Thailand	JX646801	JX646866	JX646849	N/A
Neoscytalidium dimidiatum	CBS 145.78[T]	*Homo sapiens*	United Kingdom	KF531816	KF531795	KF531796	N/A
N. dimidiatum	CBS 251.49	*Juglans regia*	USA	KF531819	KF531797	KF531799	N/A

Note: Newly generated sequences are indicated in bold.

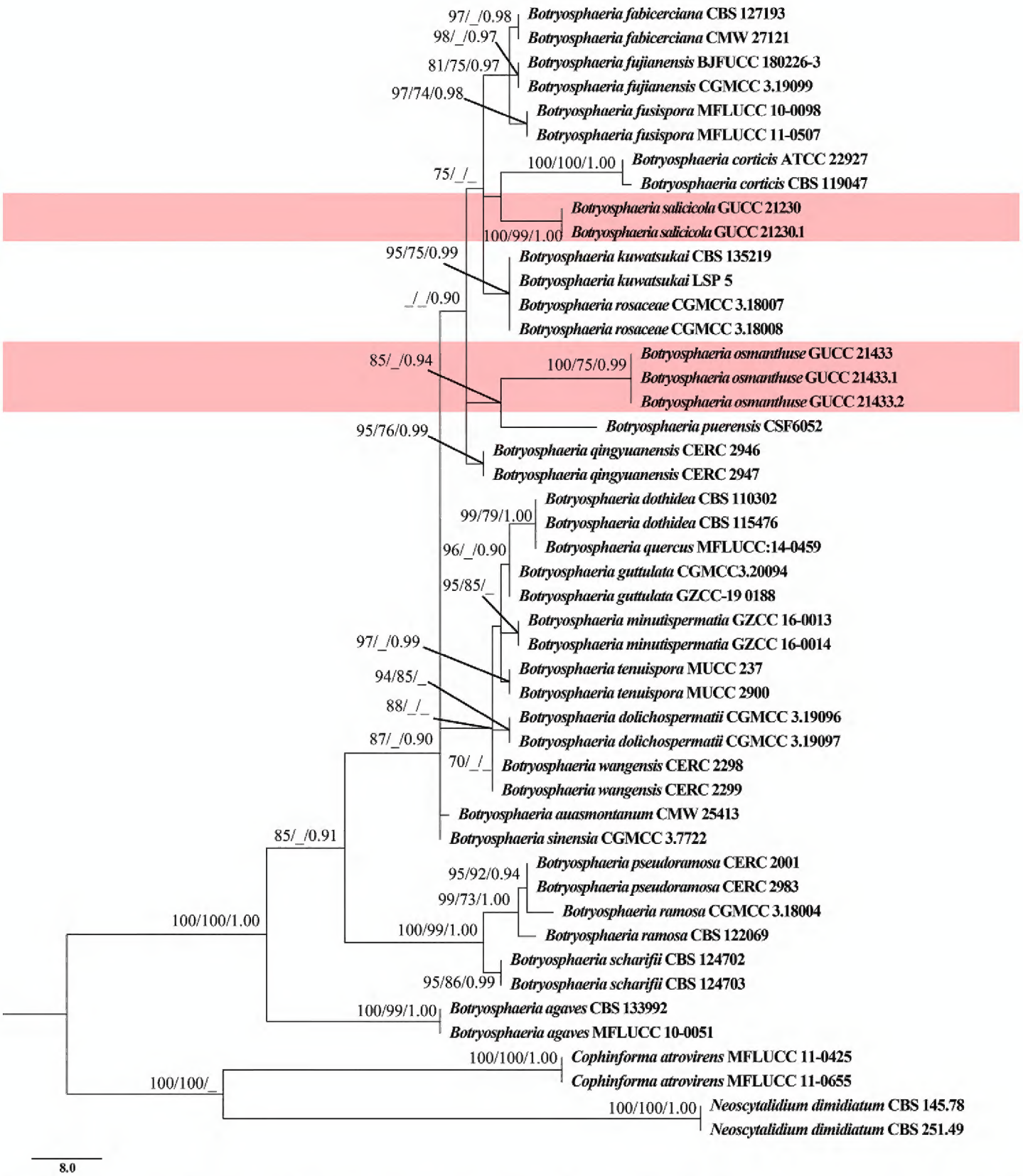

Figure 1. Trees resulting from MP analysis of the combined ITS, *rpb2*, *tef1* and *tub2* sequence alignment for forty-three isolates in *Botryosphaeria*. RAxML and MP bootstrap support values (ML, MP ≥ 70%) and Bayesian posterior probability (PP ≥ 0.90) are denoted on the nodes (ML/MP/PP). The tree was rooted to *Neoscytalidium dimidiatum* (CBS 145.78 and CBS 251.49) and *Cophinforma atrovirens* (MFLUCC 11-0425 and MFLUCC 11-0655). The new species are highlighted in pale red. The scale bar indicates 8.0 expected changes per site.

Taxonomy

Botryosphaeria salicicola **J. E. Sun, C. R. Meng & Yong Wang bis, sp. nov.**
MycoBank No: 843685
Figs 2a–i

Etymology. In reference to the host from which the fungus was first isolated.

Diagnosis. *Botryosphaeria salicicola* is characterised by oval to broadly fusiform ascospores (25.2 × 10.8; L/W = 2.3 vs. 22.7× 7.8 µm, L/W = 2.9) and cylindrical to clavate asci (65–170 × 20–30 µm), with moderate growth rate.

Type. China, Guizhou Province, Guiyang City, 26°65'N, 106°63'W, from branches of *Salix* sp., 20 June 2020, C.R. Meng, HGUP 21230 (holotype), ex-type culture GUCC 21230.

Description. Saprobic on dead branches of *Salix*. **Teleomorph:** ***Ascomata*** superficial, becoming erumpent at maturity, aggregated, thick-walled, wall composed of dark brown, thick-walled ***textura angularis***, becoming thinner-walled and hyaline towards the inner layers, 160 µm diam. ***Hamathecium*** comprising hyaline, septate, branched, 2–3.5 µm wide filamentous pseudoparaphyses. ***Asci*** 65–170 × 20–30 µm, 8-spored, bitunicate, cylindrical, to clavate, stipitate. ***Ascospores*** 22–26 × 9.0–13 µm (average = 25.2 × 10.8 µm, n = 20, L/W = 2.3), irregularly biseriate in the ascus, hyaline, guttulate, smooth with granular contents, aseptate, oval to broadly fusiform, widest in the middle or upper third of the ascospore, tapering to the obtuse base and apex. **Anamorph:** Not observed.

Culture characteristics. Ascospores germinate on PDA within 24 hours at room temperature (25 °C). Colonies with white fluffy mycelium on PDA (90 mm), after 7 days becomes grey-black at the bottom of centre, olivaceous-grey at the bottom of edge, white mycelium, raised, fluffy, dense filamentous.

Table 3. The DNA base differences in four loci between the two new species and closely-related species.

Species	Strain number	ITS (1–458 characters)	*tef1* (459–703 characters)	*tub2* (704–1039 characters)	*rpb2* (1040–1754 characters)
Botryosphaeria salicicola	GUCC 21230	0	0	0	–
	GUCC 21230.1	0	0	0	–
B. corticis	CBS 119047	10 (gap: 2)	11 (gap: 6)	6 (gap: 0)	–
	ATCC 22927	10 (gap: 2)	11 (gap: 6)	6 (gap: 0)	–
B. fabicerciana	CBS 127193	4 (gap: 3)	8 (gap: 2)	3 (gap: 1)	–
	CMW 27121	4 (gap: 3)	8 (gap: 2)	3 (gap: 1)	–
B. fujianensis	CGMCC 3.19099	4 (gap: 3)	8 (gap: 2)	4 (gap: 1)	–
	BJFUCC 180226-3	4 (gap: 3)	8 (gap: 2)	4 (gap: 1)	–
B. fusispora	MFLUCC 10-0098	4 (gap: 3)	10 (gap: 3)	3 (gap: 1)	–
	MFLUCC 11-0507	4 (gap: 3)	10 (gap: 3)	3 (gap: 1)	–
B. kuwatsukai	CBS 135219	4 (gap: 4)	7 (gap: 2)	–	–
	LSP 5	4 (gap: 4)	7 (gap: 2)	–	–
B. rosaceae	CGMCC 3.18007	4 (gap: 4)	7 (gap: 2)	2 (gap: 0)	–
	CGMCC 3.18008	4 (gap: 4)	7 (gap: 2)	2 (gap: 0)	–
B. dothidea	CBS 115476	8 (gap: 2)	12 (gap: 4)	3 (gap: 1)	–
	CBS 110302	8 (gap: 2)	12 (gap: 4)	3 (gap: 1)	–
Species	**Strain number**	**ITS (1–456 characters)**	***tef1* (471–702 characters)**	***tub2* (703–1034 characters)**	***rpb2* (1035–1750 characters)**
Botryosphaeria osmanthuse	GUCC 21443	0	0	0	0
	GUCC 21443.1	0	0	0	0
	GUCC 21443.2	0	0	0	0
B. puerensis	CSF6052	1 (gap: 1)	13 (gap: 4)	8 (gap: 0)	8 (gap: 0)
B. dothidea	CBS 115476	5 (gap: 1)	9 (gap: 2)	12 (gap: 0)	–
	CBS 110302	5 (gap: 1)	9 (gap: 2)	12 (gap: 0)	–

Figure 2. *Botryosphaeria salicicola* (GUCC 21230, holotype) **a–c** ascomata on natural substrate **d** section through ascomata **f** mature asci **g** ascospores **h** colony on PDA (left: above, right: reverse). Scale bars: 400 μm (**b**); 200 μm (**c**); 50 μm (**d**); 40 μm (**e**); 20 μm (**f, g**); 15 mm (**h**).

Distribution. China, Guizhou Province, Guiyang City.

Other material examined. China, Guizhou Provice, Guiyang City, 26°65'N, 106°63'W, from dead branches of *Salix*, 20 June 2020, C.R. Meng, HGUP 21230, living culture GUCC 21230.1.

Notes. NCBI BLAST searches of ITS sequences from our strains suggested a high degree of similarity (99–100%) to *Botryosphaeria dothidea*. However, *B. salicicola* and

B. dothidea show distant phylogenetic relationships in the phylogeny. *Botryosphaeria salicicola* has longer asci (65–170 × 20–30 µm vs. 63–125 × 16–20 µm) than *B. dothidea* and longer ascospores (25.2 × 10.8; L/W = 2.3 vs. 22.7× 7.8 µm, L/W = 2.9) (Slippers et al. 2004). The phylogenetic analyses indicate that *Botryosphaeria salicicola* forms an independent branch with respect to *B. corticis*, *B. fabicerciana*, *B. fusispora*, *B. fujianensis*, *B. kuwatsukai* and *B. rosaceae*. Comparing the morphological characteristics shows that *B. corticis* has longer ascospores than *B. salicicola* (29.3 × 11.6 µm vs. 25.2 × 10.8 µm) (Phillips et al. 2006); *B. fusispora* has shorter asci than *B. salicicola* (77.5–112.5 × 20–25 µm vs. 65–170 × 20–30 µm) (Liu et al. 2012); *B. rosaceae* has longer ascomata than *B. salicicola* (170–290 µm vs. 160 µm) (Zhou et al. 2017). The sexual morphs of *B. fabicerciana* (Chen et al. 2011), *B. fujianensis* (Chu et al. 2021) and *B. kuwatsukai* (Xu et al. 2015) are unknown.

Botryosphaeria osmanthuse **J. E. Sun, C. R. Meng & Yong Wang bis, sp. nov.**
MycoBank No: 843684
Figs 3a–i

Etymology. In reference to the host from which the fungus was first isolated.

Diagnosis. *Botryosphaeria osmanthuse* is characterised by aseptate narrowly fusiform conidia (16.0–20.5 × 5.0–6.0 µm (average = 17.0 × 5.3 µm, n = 45, L/W = 3.2) and short-length conidiogenous cells (8.5–10.5 × 2.3–2.8 µm), with moderate growth rate.

Type. China, Guangxi Province, Nanning City, 22°51'N, 108°19'E, from leaves of *Osmanthus fragrans*, 20 October 2017, C.R. Meng, HGUP 21433 (holotype), ex-type living culture GUCC 21433.

Description. Saprobic on living leaves of *Osmanthus fragrans*. **Teleomorph:** Not observed. **Anamorph:** ***Conidiomata*** up to 200 µm diam., covered with hyphae, black, globose, ostiolate, solitary, separate, uniloculate, immersed to semi-immersed. ***Conidiomatal wall*** composed of thick-walled, dark brown cells of ***textura angularis***, becoming thin-walled and hyaline towards the inner region. ***Conidiophores*** reduced to conidiogenous cells. ***Conidiogenous cells*** 8.5–10.5 × 2.3–2.8 µm (average = 10 × 2.5 µm, n = 20), holoblastic, discrete, hyaline, cylindrical to lageniform, phialidic with periclinal thickening. ***Paraphyses*** not were seen. ***Conidia*** 16.0–20.5 × 5.0–6.0 µm (average = 17.0 × 5.3 µm, n = 45, L/W = 3.2), hyaline, thin-walled, smooth with granular contents, unicellular, aseptate narrowly fusiform, base subtruncate to bluntly rounded.

Culture characteristics. Conidia germinate on PDA within 24 hours at room temperature (25 °C) with germ tubes produced from both ends of the conidia. Colonies with white fluffy mycelium on PDA (90 mm), after 7 days becomes raised, fluffy, white mycelium, dense filamentous.

Distribution. China, Guangxi Province, Nanning City.

Other material examined. China, Guangxi Province, Nanning City, 22°51'N, 108°19'E, from living leaves of *Osmanthus fragrans*, 20 October 2017, C.R. Meng, HGUP 21433, living culture GUCC 21433.1 and GUCC 21433.2.

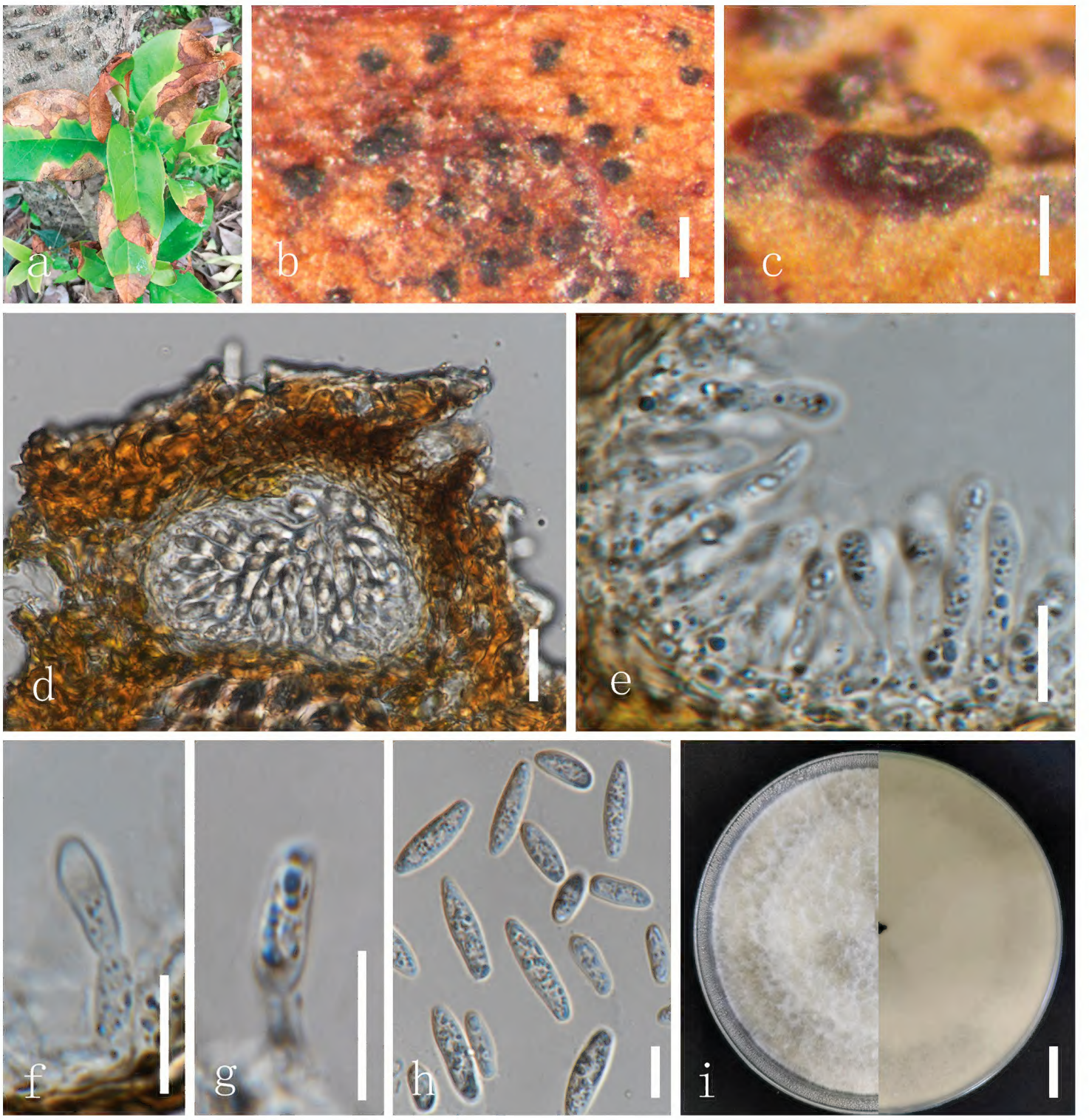

Figure 3. *Botryosphaeria osmanthuse* (GUCC 21433, holotype) **a–c** colonies on natural substrate **d** section through conidiomata **e–g** conidiophores and conidia **h** conidia **i** colony on PDA (left: above, right: reverse). Scale bars: 300 μm (**b**); 140 μm (**c**); 50 μm (**d**); 20 μm (**e**); 10 μm (**f–h**); 15 mm (**i**).

Notes. NCBI BLAST searches of ITS sequences from our strains suggest a high degree of similarity (99–100%) to *Botryosphaeria dothidea*. However, DNA bases in the two loci (*tef1* and *tub2*) showed a high amount of difference between *B. osmanthuse* and *B. dothidea*. *Botryosphaeria osmanthuse* shows close phylogenetic affinity to *B. puerensis* (Fig. 1). Comparing the morphological characteristics, conidia of *B. osmanthuse* (av. 17.0 × 5.3; L/W = 3.2) are narrower and shorter than *B. puerensis* (av. 26.8 × 6.4; L/W = 4.2) (Li et al. 2020). *Botryosphaeria osmanthuse* was first isolated from *Osmanthus fragrans* (Oleaceae), while *B. puerensis* has been reported from *Eucalyptus urophylla* (Myrtaceae).

Discussion

Two new species of *Botryosphaeria*, *B. salicicola* and *B. osmanthuse*, are described and illustrated from southern China in this paper. Previously reported *Botryosphaeria* species in China are listed in Table 4. Thirteen *Botryosphaeria* species were described from nine different areas in southern China, covering three climatic zones (northern sub-tropical zone, central sub-tropical zone and warm temperate zone) along an altitudinal gradient (Hui 2021). Most species, such as *B. fabicerciana*, *B. fujianensis*, *B. fusispora*, *B. kuwatsukai*, *B. dolichospermatii*, *B. minutispermatia*, *B. pseudoramosa*, *B. qingyuanensis* and *B. wangensis*, often caused serious diseases on their hosts (Xu et al. 2015; Ariyawansa et al. 2016; Zhou et al. 2016, 2017; Li et al. 2018, 2020; Vu et al. 2019; Chen et al. 2020; Chu et al. 2021). Geographical and climatic regions have a large influence on the taxonomy, ecological distribution and pathogenicity of *Botryosphaeria* species (Phillips et al. 2013).

Table 4. List of Chinese *Botryosphaeria* strains.

Species	Strain	Host/ Natural substrate	Regions	Fungi	References
Botryosphaeria fabicerciana	CBS 127193	*Eucalyptus* sp.	Fujian	Pathogens	Li et al. (2018)
	CMW 27094	*Eucalyptus* sp.	Fujian	Pathogens	Li et al. (2018)
	CMW 27121	*Eucalyptus* sp.	Fujian	Pathogens	Li et al. (2018)
	CERC 2930	*Eucalyptus* sp.	Yunnan	Pathogens	Li et al. (2018)
	CERC 3446	*Eucalyptus* sp.	Guangdong	Pathogens	Li et al. (2018)
	CERC 2912	*E. urophylla* & *E. grandis*	Yunnan	Pathogens	Li et al. (2018)
	CERC 2913	*E. urophylla* & *E. grandis*	Yunnan	Pathogens	Li et al. (2018)
B. fujianensis	CGMCC 3.19099	*Vaccinium uliginosum*	Fujian	Pathogens	Chu et al. (2021)
	BJFUCC 180226-3	*V. uliginosum*	Fujian	Pathogens	Chu et al. (2021)
	BJFUCC 180226-4	*V. uliginosum*	Fujian	Pathogens	Chu et al. (2021)
B. fusispora	CSF6063	*E. urophylla* & *E. grandis*	Yunnan	Pathogens	Li et al. (2020)
	CSF6178	*E. globulus*	Yunnan	Pathogens	Li et al. (2020)
	CSF5872	*E. urophylla* & *E. grandis*	Yunnan	Pathogens	Li et al. (2020)
	CSF5950	*E. urophylla* & *E. grandis*	Yunnan	Pathogens	Li et al. (2020)
	CSF6160	*E. globulus*	Yunnan	Pathogens	Li et al. (2020)
	CSF6056	*E. urophylla* & *E. grandis*	Yunnan	Pathogens	Li et al. (2020)
B. guttulata	CGMCC3.20094	Decaying branch	Guizhou	Saprobes	Chen et al. (2020)
	GZCC 19-0186	Decaying branch	Guizhou	Saprobes	Chen et al. (2020)
	GZCC 19-0188	Decaying branch	Guizhou	Saprobes	Chen et al. (2020)
B. kuwatsukai	CBS 135219	*Malus domestica*	Unknown	Pathogens	Xu et al. (2015)
	LSP 5	*Pyrus* sp.	Unknown	Pathogens	Xu et al. (2015)
B. dolichospermatii	CGMCC 3.19096	*V. uliginosum*	Fujian	Pathogens	Chu et al. (2021)
	CGMCC 3.19097	*V. uliginosum*	Fujian	Pathogens	Chu et al. (2021)
	GZCC 16-0013	Dead wood	Guizhou	Saprobes	Ariyawansa et al. (2016)
	GZCC 16-0014	Dead wood	Guizhou	Saprobes	Ariyawansa et al. (2016)
B. pseudoramosa	CERC 2001	*E. hybrid*	Guangxi	Pathogens	Li et al. (2018)
	CERC 2982	Unknow	Guangxi	Pathogens	Li et al. (2018)
	CERC 2983	*Melastoma sanguineum*	Guangxi	Unsure	Li et al. (2018)
	CGMCC 3.18739	*Eucalyptus* sp.	Guangxi	Unsure	Li et al. (2018)
	CERC 3462	*Eucalyptus* sp.	Guangxi	Unsure	Li et al. (2018)
	CERC 2019	*E. urophylla* & *E. grandis*	Guangxi	Unsure	Li et al. (2018)
	CERC 2987	*Me. sanguineum*	Guangxi	Unsure	Li et al. (2018)
	CERC 3455	*Eucalyptus* sp.	Guangxi	Unsure	Li et al. (2018)
	CERC 2988	*Me. sanguineum*	Guangxi	Unsure	Li et al. (2018)

Species	Strain	Host/ Natural substrate	Regions	Fungi	References
B. qingyuanensis	CERC 2946	*E. hybrid*	Guangdong	Pathogens	Li et al. (2018)
	CERC 2947	*E. hybrid*	Guangdong	Pathogens	Li et al. (2018)
B. ramosa	CGMCC 3.18004	*Acacia* sp.	Hainan	Unsure	Vu et al. (2019)
	CGMCC 3.18006	*Myrtaceae*	Guangdong	Unsure	Vu et al. (2019)
B. rosaceae	CGMCC 3.18007	*Malus* sp.	Shandong	Unsure	Zhou et al. (2017)
	CGMCC 3.18008	*Amygdalus* sp.	Shandong	Unsure	Zhou et al. (2017)
	CGMCC3.18009	*Malus* sp.	Shandong	Unsure	Zhou et al. (2017)
	CGMCC3.18010	*Pyrus* sp.	Shandong	Unsure	Zhou et al. (2017)
	CFCC 82350	*Malus* sp.	Unknown	Unsure	Zhou et al. (2017)
	CGMCC3.18011	*Pyrus* sp.	Shandong	Unsure	Zhou et al. (2017)
B. sinensia	CGMCC 3.17722	*Populus* sp.	Henan	Unsure	Zhou et al. (2016)
	CGMCC 3.17723	*Morus* sp.	Henan	Unsure	Zhou et al. (2016)
	CGMCC 3.17724	*Juglans regia*	Henan	Unsure	Zhou et al. (2016)
	CFCC 82346	*J. regia*	Beijing	Unsure	Zhou et al. (2016)
	CFCC 82255	*Ma. pumila*	Beijing	Unsure	Zhou et al. (2016)
B. wangensis	CERC 2298	*C. deodara*	Henan	Pathogens	Li et al. (2018)
	CERC 2299	*C. deodara*	Henan	Pathogens	Li et al. (2018)
	CGMCC 3.18744	*C. deodara*	Henan	Pathogens	Li et al. (2018)
	CERC 2300	*C. deodara*	Henan	Pathogens	Li et al. (2018)
	CSF5820	*E. urophylla* & *E. grandis*	Yunnan	Pathogens	Li et al. (2020)
	CSF5733	*Eucalyptus* sp.	Yunnan	Pathogens	Li et al. (2020)
	CSF5944	*E. urophylla* & *E. grandis*	Yunnan	Pathogens	Li et al. (2020)
	CSF5971	*E. urophylla* & *E. grandis*	Yunnan	Pathogens	Li et al. (2020)
	CSF5781	*E. globulus*	Yunnan	Pathogens	Li et al. (2020)
	CSF6174	*E. globulus*	Yunnan	Pathogens	Li et al. (2020)
	CSF5737	*Eucalyptus* sp.	Yunnan	Pathogens	Li et al. (2020)
B. archontophoenicis	HKU (M) 3539	*Archontophoenix alexandrae*	Hong Kong	Saprobes	Index Fungorum and mycobank
B. brunneispora	HKU (M) 3987	*Trachycarpus fortune*	Hubei	Unsure	Index Fungorum and mycobank
B. cunninghamiae	N/A	*Cunninghamia lanceolata*	China	Saprobes	Index Fungorum and mycobank
B. puerensis	HMAS 255719	*E. urophylla* & *E. grandis*	China	Pathogens	Index Fungorum and mycobank
B. qinlingensis	BJFC S1576	*Quercus aliena var. acuteserrata*	Shaanxi	Unsure	Index Fungorum and mycobank
B. yedoensis	N/A	*Prunus yedoensis*	Taiwan	Unsure	Index Fungorum and mycobank

Botryosphaeria species have been known to exist in many woody plants (Crous et al. 2006; Liu et al. 2012; Phillips et al. 2013; Ariyawansa et al. 2016; Dissanayake et al. 2016; Slippers et al. 2017). *Botryosphaeria dothidea*, the type species of the genus (Slippers and Wingfield 2007), is known from numerous hosts (Phillips et al. 2013; Marsberg et al. 2017) and was isolated from an *Asphondylia* gall on *Lamiaceae* in Italy and Poland (Zimowska et al. 2020). Other species of *Botryosphaeria* have often been isolated from many wood plants (Table 2). Amongst them, *B. fabicerciana*, *B. fusispora*, *B. kuwatsukai*, *B. pseudoramosa*, *B. rosaceae*, *B. wangensis* and *B. puerensis* often exist in many economic plants, such as *Eucalyptus* sp., *Pyrus* sp., *Malus* sp., *Citrus* sp. and *Vaccinium* sp. (Phillips et al. 2006; Lazzizera et al. 2008; Zhou et al. 2017; Li et al. 2018, 2020; Chen et al. 2020). Our strains were isolated from the *Salix* (Salicaceae) and *O. fragrans* (Oleaceae) of woody plants. In contrast, the few hosts or natural substrates of the known species belong to the Salicaceae and Oleaceae.

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